Chemical Stimulation of Host Tree Antibiosis for Southern Pine Beetle, *Dendroctonus frontalis* Zimm., Suppression

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INTRODUCTION

The southern pine beetle (SPB), Dendroctonus frontalis Zimmermann, is responsible for most insect-caused mortality of timber in the South. Periodic epidemics produce timber and pulpwood losses valued in the millions (Price and Doggett 1978). Current methods for slowing or stopping SPB spots--cut and leave, use of insecticides, pile and burn, or intensive salvage (Swain and Remion 1981)--are limited by cost of chemicals, labor and equipment, or environmental concerns about pesticides (Billings 1980). Due to the potential hazard to humans, livestock, beneficial insects, fish, and wildlife (Bennett and Ciesla 1971), there will likely be future restrictions on use of chemicals. Alternative methods for protecting high value trees from SPB in an economical and environmentally sound manner are needed.

Induced host plant resistance may have potential as an alternative method for SPB suppression. Although resistance to pests and pathogens has been induced by chemical treatment of some plants, a response against insects has not previously been reported in treated trees (Stipes 1988). A formulation of 32.7 percent sodium N-methyldithiocarbamate (SMDC) in 99.9 percent dimethylsulfoxide (DMSO) (4:1 v/v) applied by hack and squirt appears effective against SPB (Roton 1987). This formulation induces a hypersensitive response in which long, vertical, necrotic lesions are formed. The response may change monoterpene composition, does not kill trees when properly applied, and is apparently not harmful to natural enemies (Roton 1987). SMDC is a soil fumigant for control of fungi, bacteria, nematodes, weeds, and soil insects (Thomson 1986). DMSO is an agricultural solvent, penetrant, carrier, and antiviral agent (Smale 1969).

The method of introducing the chemical into the target tree may influence treatment efficacy and acceptance. Infusion is less contaminating than spraying, requires less labor and equipment, and relies instead on atmospheric pressure and the tree's uptake/translocation capability (Stipes 1988). The research described here shows the residual life of SMDC and tests the ability of SMDC+DMSO infusion to inhibit SPB brood production. Also reported here is a preliminary evaluation of SMDC+DMSO infusion on SPB ratio of increase (Thatcher and Pickard 1964), a measure of the success of SPB development in individual trees.

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MATERIALS, METHODS, AND RESULTS

Residue Tests. In the fall of 1986, active SPB spots in Stewart County, Georgia, were used to evaluate residual activity of the test compound[1]. Four replications, each of 32.7 percent SMDC in DMSO (4:1 v/v) in hacks, bark hacks only, and no treatments (control), were applied to loblolly pines (*Pinus taeda* L.). The chemical (5.0 - 10.0 ml) was applied to 2.5 cm long by 2.0 cm deep axe frills made around the circumference of the tree at 1.0 m above the ground and bridged by 2 to 4 cm of undamaged bark. Uninfested trees were sampled 1.0 m above the infusion point, at midbole, and 1.0 m below the live crown. The outer xylem, phloem, and bark were split off from small portions of the trunk at the designated height. The bark was peeled away, phloem separated from xylem and cut into small pieces. The upper 1.0 cm of xylem was turned into wood shavings. Samples were immediately processed, frozen, and stored in freezers.

Extracts were made from the samples for analysis by gas chromatography (GC) for methylisothiocyanate (MIT), the breakdown product of SMDC. A 5-10 g sample of xylem shavings or phloem bits sat overnight covered with water at room temperature in tared liquid scintillation vials. The aqueous portion was salted, 5.0 ml of ethyl acetate added, and then gently shaken for an hour. The organic layer containing MIT was drawn off and dried with sodium sulfate. GC analysis was performed on a capillary column at 180 °C with a nitrogen-phosphorous detector at 275 °C. Residual chemical was quantified by comparison of integrator counts of samples to counts from an analytical standard curve. Total nanograms of MIT was obtained by linear regression of emergence on dose per application point and expressed as ppm MIT, sample dry weight.

In spring 1987, eight pines were treated with chemical and sampled for residues as in 1986. Samples were taken 1, 4, 7, and 14 days posttreatment. The test was repeated in late summer, deleting sample day 4 and adding day 21.

In spring 1988, six trees were treated with chemical. One tree was intensively sampled at 3 heights (low, mid, top) as described. Three replicates at each height of phloem strips and drilled xylem shavings were removed and placed on dry ice. Day 1 samples were taken at 1, 5, 10, and 24 hours. Subsequent samples were taken on day 4 and weeks 1, 2, 4, 8, 10, 12, 14, and 20. In addition, infusion sites were sampled on weeks 2, 4, 8, 10, 12, and 14. All residues were determined as described above.

[1]Berisford, C. W. and M. J. Dalusky. 1989. Efficacy of systemic toxicants for southern pine beetle control. Final Report, U.S. Department of Agriculture, Forest Service, SFES Cooperative Agreement #19-87-097. 11 p., 2 tables, 9 figures. 1989.

Results. The 1988 SMDC+DMSO mobility study (fig. 1) confirmed results from 1986 and 1987. No residues were detected above the low height samples. Residual MIT, detected in the xylem at 1.0 m above the application point at 5 hours posttreatment, was undetectable by day 28. Ploem residues were detected at 1.0 m at 10 hours, peaked at 24 hours, then tailed off. No residues were detected from application sites after day 21 through day 140. The streaking effect on the xylem extended to midbole by day 14 and into the upper bole by week 4. The absence of residues after 21 days suggests that the infused formulation may metabolize within, or volatilize from, the tree.

Efficacy tests with hanging bolts, 1987. Loblolly pines at the head of active SPB infestations (spots) were treated in Camp Livingston, Catahoula Ranger District, Kisatchie National Forest, Louisiana. From 1984-1987, 5.0 ml of chemical were placed in horizontal, 1.0 - 5.0 mm deep X 75 mm long, axe frills, 1.0 m above the ground. Six unattacked trees were felled on the same day 1.3, 12, 18, 20, 27.5, and 28 months posttreatment and each cut into four 150 cm lengths. Four bolts, each from the 12-, 20-, and 27.5-month trees were moved to Dawson County, Georgia, and, with control bolts, were hung from trees at the front of an active SPB spot (Berisford and others 1980). Trees supporting bolts were baited with frontalure to ensure attack. Bolts from the remaining trees and an untreated control tree were installed at the advancing edge of a SPB spot in Rapides Parish, Louisiana.

When SPB became pupae or callow adults, 41.0 cm sections of these bolts were caged to collect emerging beetles. When emergence ceased, gallery lengths were measured and oviposition estimates (eggs/m^2) calculated (Foltz and others 1976).

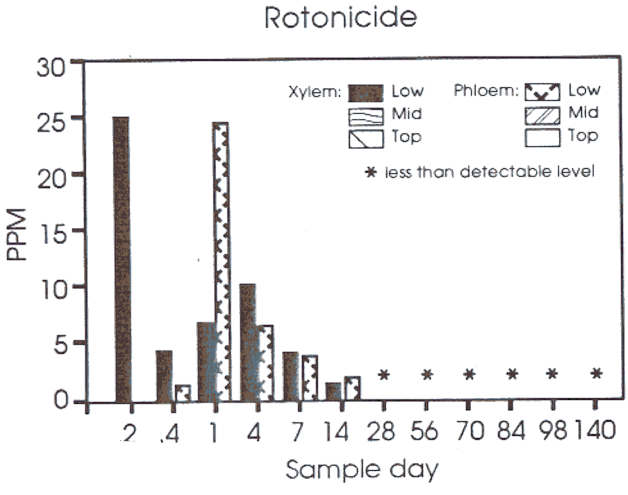

Figure 1.--*Residues of SMDC (MIT) detected in the xylem and phloem of treated pines at 1.0 m above the infusion point, spring - fall 1988.*

Results. In Louisiana, brood emergence from treated bolts was 58 percent less than controls. In addition, there was 54 percent less emergence from untreated bolts than support trees (fig. 2). There was only 3 percent variation in emergence from treated tree bolts. Estimated egg densities were similar in treated and check bolts, but fewer eggs were found in treated and check bolts than in support trees (fig. 2). There was less survival in treated bolts (fig. 3) and less survival with longer treatment-attack intervals. The Georgia replicates were misplaced, and it was not possible to analyze the data due to lack of experimental error. However, the emergence counts from each treatment were similar to those seen in the Louisiana test.

There was a consistent reduction in SPB emergence in Louisiana. Apparently SMDC+DMSO reduces brood production indirectly by eliciting a hypersensitive response. Fewer SPB emerged from trees treated from 1.3 to 28 months before felling. Therefore, the induced response can last more than 2 years. Lower survival in check bolts than support trees is probably due to desiccation of cut bolts.

Dose response/efficacy tests. Tests were conducted in active SPB spots consisting of 12 to 27 infested trees in the Bienville Ranger District, Bienville National Forest, Mississippi. From November 16-18, 1988, five unattacked trees at the head of each of six active SPB spots were randomly assigned to four treatments and a control. Chemical (3.0, 6.0, 9.0, or 12.0 ml) or water (12.0 ml) were

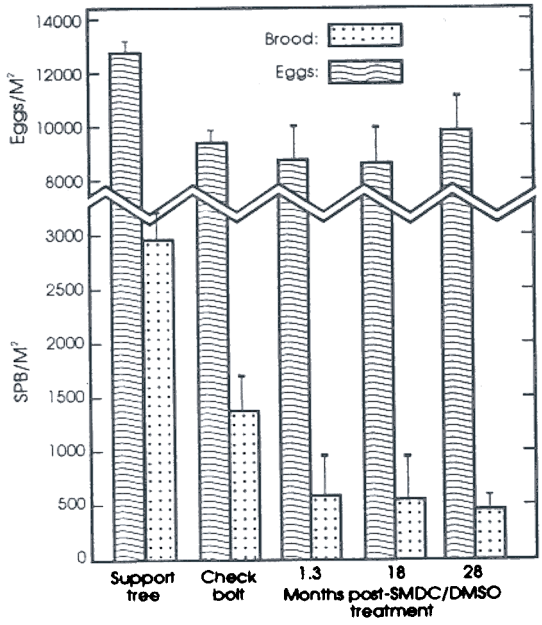

Figure 2.--*Southern pine beetle development; estimated oviposition and emergence counts from bolts from treated trees, untreated control bolts, and from bolts taken from support trees. Gates = + 2 SE.*

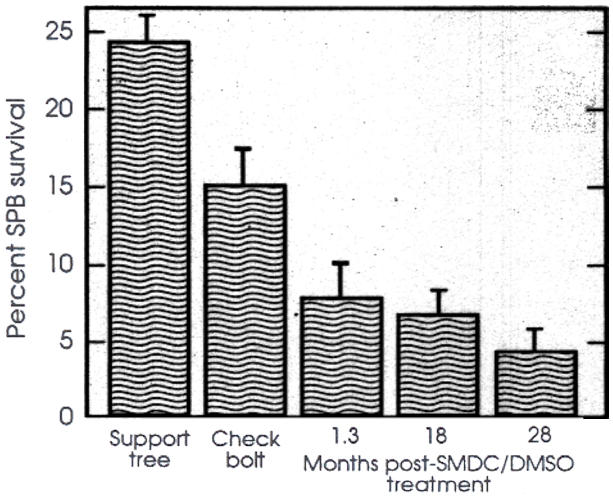

Figure 3.--*Percent survival of southern pine beetle in bolts taken from support trees, untreated check trees, and SMDC + DMSO-treated. Gates = + 2 SE.*

pipetted into horizontal axe frills, as described above. On February 21, 1989, frontalure was placed on trees to ensure SPB attack.

Bark samples (ca. 30 cm long) were removed from the circumference of the bole at 3.7 and 6.1 m when SPB reached the pupa/callow adult stage. Eight disks (94 cm^2) were removed from each 30-cm long sample for radiography, each placed in a rearing cup, and emerging SPB were counted. Data were analyzed for each sample height with polynomial regressions of emergence count means and weighted with the reciprocal of the variance. Significant regression of emergence on dose per application point was plotted to produce a dose-response line with 95 percent C.I.

Five loblolly pines (*Pinus taeda* L.) in Camp Livingston, Catahoula Ranger District, Kisatchie National Forest, Louisiana, were treated on May 23, 1989. The bark was shaved smooth, and 2.2-cm holes were drilled on 15.24-cm centers around the circumference of each tree. Coiled Tampax® tampons were pushed into the holes and tamped even with the bark surface. Each tree received either 2.5, 5.0, or 10.0 ml SMDC+DMSO/hole injected through the center of the coiled plugs. There were two 10.0 ml/hole treatment trees in each block, control trees received 10 ml of water/hole, and infusion sites were wrapped with two layers of duct tape. The same method was used to infuse trees at the head of active southern pine beetle infestations of 40 or more trees on the Yellowpine Ranger District, Sabine National Forest, Hemphill, Texas, on May 30, and on the Neches Ranger District, Davy Crockett National Forest, Ratcliff, Texas, from June 5 - September 30, for a total of nine replicated trials. If SPB did not attack by posttreatment day 14, aggregation pheromone (frontalin: a-pinene 1:2) was applied to ensure attack. The data were processed as described above for the Mississippi studies.

Results. There were no significanct regression lines for emergence from Mississippi tests. While the mean response across doses and heights was fairly constant, the consistency of the response improved as dosage increased. The residuals of the observed and predicted values showed that the variance of the data decreased with increasing dose. Emergence counts were lower, and the range of the data was narrower from 6.0 and 12.0 ml/hack treatments compared with the check and other treatment trees.

In Louisiana and Texas, the regression lines for emergence from both heights were not significant when all plots were analyzed. However, when the data were tabulated, emergence was found to be high from all trees from the first three plots, in which beetle mass attack occurred 7 days posttreatment. In six later blocks, mass attack occurred between 14 and 21 days. In these blocks at the upper height, there was no significant dose-response regression. However, at the lower height, there was a significant decreasing linear response (Prob. $>F = 0.0001$, $r^2 = 0.84$) as dose increased. Confidence intervals (95 percent) showed significant reductions with increased dose. At the 10.0-ml infusion rate there was a 92-percent average reduction in SPB emergence (fig. 4).

Preliminary ratio of increase tests. Bolts (91.5 cm) were cut at 4.6 m from trees in two blocks installed on the Neches Ranger District, Davy Crockett National Forest, Ratcliffe, Texas, on June 6, 1989. Portions (41.0 cm) were excised from the larger bolts and placed in rearing cans.

Successful SPB attacks were identified and counted (Thatcher and Pickard 1964) on four bark disks (94 cm^2) cut from the lower portion of each bolt. The bark surface area of each bolt was measured, and all data were converted to counts per 0.1 m^2. The ratio of increase (RI) was calculated for each bolt using the total emergence from each bolt and the average number of successful SPB attacks from the four disk subsamples. The RI was derived from the formula (SPB

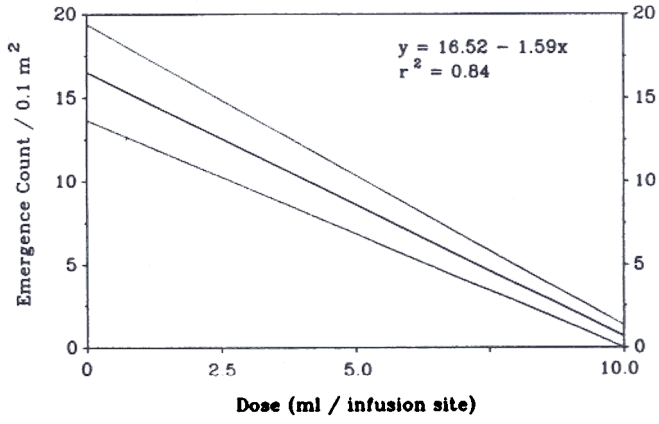

Figure 4.--*Linear regression of SPB emergence against SMDC + DMSO applied by the tampon in a hole method in six replicated in East Texas. The center line is the regression surrounded by 95-percent C.I.*

emergence) / (2 * number successful attacks) (Thatcher and Pickard 1964). Average SPB brood emergence and RI were tabulated.

Results. The ratio of increase for untreated east Texas SPB infested trees in July and August is 0.4:1 (table 1). The RI from treated trees is from 10.5- to 14-fold less than that of untreated infested trees, and corresponds with a 90 to 97 percent reduction in SPB emergence (table 1).

Infrared spectroscopy. The observed synergism of the SMDC+DMSO system prompted speculation concerning possible chemical reaction between the components. To resolve this question, the infrared spectum of an aged, SMDC+DMSO mixture was collected, and a direct subtraction of the DMSO spectrum performed. Infrared spectra of newly made SMDC and DMSO, of SMDC+DMSO, and of 3 month SMDC+DMSO mixtures, were collected with a Nicolet Fourier Transform Infrared Spectrophotometer (Model DXB-20). Samples were prepared as a smear on a KB^r pellet and data collected over 10 scans at a resolution of 4 cm^{-1}.

Results. When the resulting difference spectrum is compared with that of a fresh SMDC solution over the region 1,900 cm^{-1} to 900 cm^{-1} (fig. 5), some differences in peak intensities are readily apparent. However, the frequencies of the absorption bands are esentially identical. The slight shift to a higher frequency of the C=S peak (1,300 cm^{-1}) in the DMSO mixture may indicate some associative phenomenon between the components. There is no evidence to suggest formation of any new compound in the neat mixture.

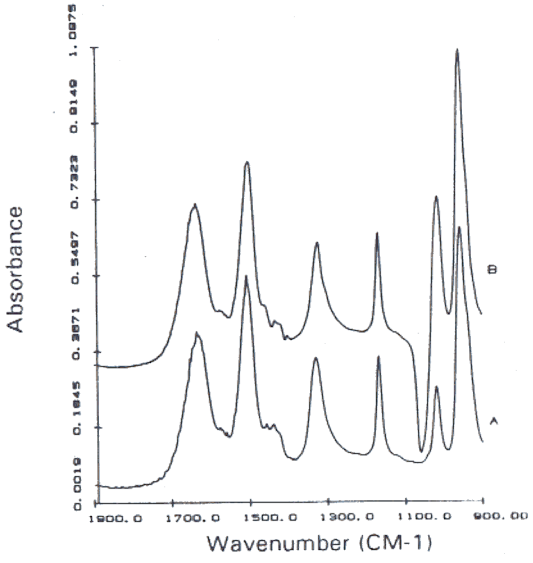

Figure 5.--*Comparison of the infrared spectrum over the region 1,900 cm^{-1} to 900 cm^{-1} of (A) a fresh SMDC solution, and (B) an aged, SMDC + DMSO mixture minus DMSO (difference spectrum).*

DISCUSSION AND CONCLUSIONS

In Georgia, mobility testing showed little or no movement of MIT above the lower bole, and then only at very low levels. Residues peaked within 3 days of application and were only present in the lower bole for 3 weeks. The residue studies do not support a SMDC+DMSO translocation hypothesis.

Table 1.--*Ratios of increase and emergence/0.1m^2 after treatment with various doses of SMDC+DMSO*

Dose	Ratio of Increase Mean	n	Emergence /0.1m^2 Mean	Percent Change
0	0.42:1	1	26.83	
2.5	0.04:1	2	1.08	-95.97
5.0	0.04:1	2	2.72	-89.86
10.0	0.03:1	4	0.77	-97.13

The induced response is at least partially responsible for inhibiting SPB brood development. Efficacy is a function of the application method and dose. In Mississippi, there was a nonsignificant reduction in emergence. In Texas, comparable doses applied with a tampon in a hole significantly reduced emergence. The Louisiana hanging bolt studies, showing reduced emergence, are supported by the Texas results. Treated trees can affect SPB emergence up to 28 months after infusion. Girdling of the tree by SPB during mass attack soon after treatment reduced efficacy.

SPB development is reduced by SMDC+DMSO infusion. The pitch-soaked lesion response is involved in inhibiting of SPB brood development. Direct toxicity of the mixture of active ingredients may not be the main source of mortality. The small amount of chemical infused into the host tree, the lack of mobility, and the short-term residues, suggest stimulation of defensive chemical production. The induced resinosis and streaking of the xylem appear to be moisture dependent. The tearing effect was present continuously under high moisture conditions in Louisiana and after heavy rainfall in Mississippi. Results are more effective as the period between SMDC+DMSO infusion and beetle attack lengthens.

Lack of available moisture for translocation of the chemical or early attack on the tree by SPB before development of pitch-soaked lesions may affect resinosis and length of lesions. Others have observed that residual MIT production from SMDC fumigation of transmission poles varies widely in different buffer solutions (Miller and Morrell 1989). Two of us (Miller and Kinn, unpublished) observed the absence of resinosis in trees treated under drought conditions and the start of resinosis 2 months later shortly after a period of heavy rainfall in the summer of 1988.

Residual MIT volatilizes at fungitoxic concentrations when wetted (Zahora and Morrell 1988). This suggests that in the living tree an active ingredient might remain immobile, or cells might not be activated, until sufficient moisture is available. The range of effects of residual MIT on living trees is not known.

Infusion of SMDC+DMSO may be an alternative to other suppression tactics. The hazard to the applicator and the user appears to be low. The LD_{50} of SMDC is 820 mg/kg, much less toxic than either Lindane, Chlorpyrifos, or Fenitrothion (Thomson 1986). DMSO assists in translocation of several insecticides, but did not markedly lower LD_{50} values of insecticides used against boll weevil (Moore and others 1970). Oligman (1965) found no skin penetration of fluorescein with less than 30 percent DMSO. DMSO may penetrate animal skin in concentrations above 40 percent (Moore and others 1970). It also has extremely low toxicity to humans, animals, and plants (Anon. 1984), and is exempted from tolerances on raw agricultural commodities when applied with certain pesticides (Federal Register 1983). DMSO toxicity is so low that it is measured in grams/kg (Wong and Reinertson 1984).

Summer is the least favorable time for SPB development, with RI in East Texas for 1960-62 remaining at less than 1:1 from June - October, and an average RI of 0.4 for June - July (Thatcher and Pickard 1964). Roton (1987) found that most associated insects were not affected by SMDC+DMSO treatment and a few increased significantly in treated trees. Our results also show that SMDC+DMSO infusion may supplement seasonal SPB mortality, especially during midsummer months. Observations of control and treated trees suggest that, though the RI during midsummer may be 0.42:1, SMDC+DMSO infusion lowers this by 10.5- to 14-fold (table 1). Therefore, induced resistance may be able to play a role in suppression of breakouts from summer cut-and-leave sites. If SMDC+DMSO infusion reduces RI in individual trees in infestations by a factor of 10, then collapse of treated spots would be expected. The treatment might also be of value from fall through spring, when ratios of increase are normally high.

This method does not rapidly reduce SPB. Studies on application methods are needed to improve consistency of results. The physiological mechanisms being stimulated and the necessary seasonal effects need to be identified to determine the biochemical basis for toxicity, the optimum time for application, and the greatest impact on beetle populations.

LITERATURE CITED

Anon. 1984. Dimethyl sulfoxide (DMSO) for pesticide formulations [Leaflet No. 326-22]. [Place of publication unknown]: Crown Zellerbach Chemical Products Division.

Bennett, W. H.; Ciesla, W. M. 1971. The southern pine beetle, [Leaflet No 49]. Washington, DC: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 8 p.

Berisford, C. W.; Brady, U. E.; Mizell, R. F.; [and others]. 1980. A technique for testing insecticides for long-term prevention of bark beetle attack. Journal of Economic Entomology 73: 694-697.

Billings, R. 1980. Direct Control. Chapter 10. In: Thatcher, R. C.; Searcy, J. L.; Coster, J. E.; Hertel, G. D., eds. The southern pine beetle. Tech. Bul. 1631. Washington, DC: U.S. Department of Agriculture, Forest Service. 126 p.

Federal Register. 1983. Rules and Regulations. 48(236): 54818.

Foltz, J. L.; Mayyasi, A. M.; Hain, F. P.; [and others]. 1976. Egg-gallery length relationship and within-tree analyses for the southern pine beetle, *Dendroctonus frontalis* (Coleoptera: Scolytidae). Canadian Entomology Journal 108: 341-352.

Miller, D. B.; Morrell, J. J. 1989. Stability and methylisothiocyanate production of 12 potential solid fumigants for controlling wood decay. Holzforschung 43: 191-194.

Moore, R. J., Jr., Taft, H. M.; Payne, L. B. 1970. Dimethyl sulfoxide as a possible synergist for selected insecticides against boll weevil. Journal of Economic Entomology 63: 1342-1343.

Oligman, A. M. 1965. Topical pharmacology and toxicology of dimethyl sulfoxide - Part I. Journal of the American Medical Association 193: 140-148.

Price, T. S.; Doggett, C. 1978. A history of the southern pine beetle outbreaks in the southeastern United States. Macon, GA: Georgia Forestry Commission. 31 p.

Roton, L. M. 1987. Promising treatment for southern pine beetles. American Papermaker. October: 30-32.

Smale, B. C. 1969. DMSO, agricultural solvent, penetrant-carrier, antiviral agent. The Sulfur Institute Journal 5: 2-6.

Stipes, R. J. 1988. Glitches and gaps in the science and technology of tree injection. J. Arboric. 14: 165-172.

Swain, K. M.; Remion, M. C. 1981. Direct control methods for the southern pine beetle. Agric. Handb. 575. Washington, DC: U.S. Department of Agriculture. 15 p.

Thatcher, R. C.; Pickard, L. S. 1964. Seasonal variations in activity of the southern pine beetle in East Texas. Journal of Economic Entomology 57: 840-842.

Thomson, W. T. 1986. Agricultural chemicals - Book III. Miscellaneous chemicals; fumigants, growth regulators, repellents, and rodenticides. Fresno, CA.: Thomson Publishing. 187 p.

Wong, L. K.; Reinertson, E. L. 1984. Clinical considerations of dimethyl sulfoxide. Iowa State Veterinarian 46: 89-95.

Zahora, A. R.; Morrell, J. J. 1988. Decomposition of methylisothiocyanate in Douglas-fir heartwood. Forest Products Journal 38: 46-52.